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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Gardona

FROM: Esther Rinde, Ph.D. E. Rinde 2/12/88
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: George LaRocca
Product Manager #15
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on Oct. 22, 1987 to discuss and evaluate the weight-of-the-evidence on Gardona with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Theodore M. Farber — Theodore M. Farber

William L. Burnam — W. L. Burnam

Reto Engler — Reto Engler

Marion Copley — Marion Copley

Judith Hauswirth — Judith Hauswirth

Richard Levy/C.J. Nelson — Richard Levy

Esther Rinde — Esther Rinde

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Mike Ioannou — Mike Ioannou

Albin Kocialski — Albin R. Kocialski

Bernice Fisher — Bernice Fisher

TS-7 RECD 2/12 RLC SH

A. 3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton _____

Richard Hill/Don Barnes _____

Robert Beliles _____

Diane Beal _____

Jack Quest _____

Kerry Dearfield _____

4. Other Attendees:

Larry Reiter (OPTS) was also present.

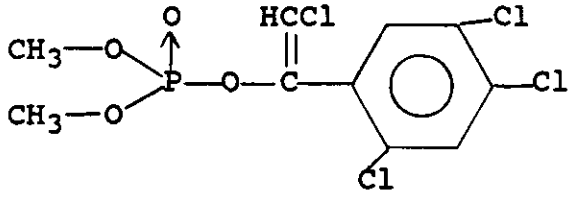
B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Ioannou; Tables and statistical analyses by B. Fisher. The material reviewed is attached to the file copy of this report.

C. Background Information:

Gardona [Tetrachlorovinphos: 2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate] is an organophosphorous insecticide developed by Shell Chemical Company, currently used mainly for the control of pests on livestock under the trade name Rabon.

Structure of Gardona:



D. Evaluation of Oncogenicity Evidence for Gardona:

1. Mouse Oncogenicity Study - Hazleton

Reference: Shell Oil "103 week chronic feeding study in mice" at Hazleton Laboratories, Unpublished study No. 776-118, June, 1980.

Gardona was administered in the diet to groups of 80 male and 80 female B6C3F1 mice at 0, 17.5, 64, 320, 1600, 8000, or 16,000 ppm for 103 weeks. Control groups (0 ppm) consisted of 160 mice/sex.

In male mice, there was a statistically significant increase in hepatocellular adenoma/carcinoma, combined at 16,000 ppm (HDT) with a statistically significant trend. In female mice, there was a statistically significant increase in hepatocellular carcinomas at 8000 and 16,000 ppm, and in combined adenoma/carcinoma at 1600, 8000, and 16,000 ppm; adenomas were statistically increased at 16,000 ppm, only; there was a statistically significant trend for adenoma, carcinoma and for combined adenoma/carcinoma, as well. Tumor incidences are given in Tables 1-3.

In males only, there was also a statistically significant increase in renal adenoma, carcinoma and adenoma/carcinoma, combined, at 16,000 ppm; there was also a statistically significant trend for adenoma, carcinoma and for combined adenoma/carcinoma, as well.

(Continued)

TABLE 1

Hazleton Mouse Study

Female Liver Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver Tumors	Dietary Concentration (ppm) 0	17.5	64	320	1600	8000	16,000	Historic. Controls Mean	Range
Carcinoma	1/119 (1)**	0/58 (0)	0/69 (0)	0/70 (0)	4/69 (6)[b]	5/66 (8)*	5/68 (7)*	(4)	(0-15)
Adenoma	0/113 (0)**	1/57 (2)	1/57 (2)[a]	0/59 (0)	1/57 (2)	2/56 (4)	3/58 (5)*	(4)	(0-18)
Adenoma and/ or Carcinoma	1/119 (1)**	1/58 (2)	1/69 (1)	0/70 (0)	5/69 (7)*	7/66 (11)**	8/68 (12)**	(8)	(0-20)

[b]First carcinoma appeared at week 55 in the 1600 ppm group.
[a]First adenoma appeared at week 79 in the 64 ppm group.
†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor).

TABLE 2

Hazleton Mouse Study
Male Liver Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver Tumors	Dietary Concentrations (ppm) 0	17.5	64	320	1600	8000	16000	Historic. Controls Mean	Range
Carcinoma	26/113 (23)	17/58 (29)	16/58 (28)	10/51 (20)	14/55 (25)	13/60 (22)	22/59 (37)[b]	(21)	(8-36)
Adenoma	2/80 (2)[a]	1/37 (3)	0/42 (0)	0/35 (0)	1/39 (3)	5/47 (11)	3/47 (6)	(10)	(0-24)
Adenoma and/ or Carcinoma	28/113 (25)*	18/58 (31)	16/58 (28)	10/51 (20)	15/55 (27)	18/60 (30)	25/59 (42)*	(31)	(16-68)

[b]First carcinoma appeared at week 66 in the 16000 ppm group.
[a]First adenoma appeared at week 99 in the control group.
†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor).

Note: Significance of trend denoted at control. Significance of pairwise comparisons with control denoted at Dose level.

*p <0.05. **p <0.01

TABLE 3

Hazleton Mouse Study

Male Kidney Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentation (ppm)							Histor. Controls	
Kidney Tumors	0	17.5	64	320	1600	8000	16000	Mean	Range
Carcinoma	0/71 (0)**	0/37 (0)	0/39 (0)	0/31 (0)	1/36 (3)[b]	1/47 (2)	9/46 (20)**	(0.2)	(0-2)
Adenoma	0/113 (0)**	0/58 (0)	0/68 (0)	0/62 (0)	1/65 (2)	0/70 (0)	4/69 (6)[a]*	(0.3)	(0-2)
Adenoma and/ or carcinoma	0/113 (0)**	0/58 (0)	0/68 (0)	0/62 (0)	2/65 (3)	1/70 (1)	13/69 (19)**	(0.5)	(0-4)

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose level. Numbers in parentheses denote percent.

*p <0.05. **p <0.01

†Number of tumor bearing animals/number of animals at risk (excluding animals that died before appearance of first tumor).
[b]First carcinoma appeared at week 104 in the 1600 ppm group.
[a]First adenoma appeared at week 54 in the 16000 ppm group.

D. 1. Mouse Oncogenicity Study - Hazleton (continued)

MTD

The Committee determined that the MTD was achieved or slightly exceeded in this study at 8000 ppm, based on body weight gain depression (>15%). There was no significant histopathology at 8000 ppm, but significant histopathology at 16,000 ppm, indicative of cellular proliferation (severe liver necrosis) was present. The liver necrosis was not life-threatening, however, as survival at this dose (16,000 ppm) was actually enhanced compared to controls.

(The sponsor maintains that the MTD was exceeded at 8000 ppm, and therefore effects above 1600 are not "experimentally valid"; however, there was a statistically significant increase in the incidence of hepatatocellular combined adenoma/carcinoma in the livers of female mice, even at 1600 ppm.)

Historical Controls

The incidences of liver tumors in treated mice of both sexes were within the range reported in the literature for B6C3F1 mice (the sponsor also indicated that the incidence in concurrent female controls was low, compared to that given in the literature).

The incidence of kidney tumors in treated male mice at 16,000 ppm, for carcinoma, adenoma and for combined adenoma/carcinoma exceeded that reported in the literature for B6C3F1 male mice.

The more pertinent historical control data from the performing laboratory were not available, however, for making the secondary comparison with either of these tumor types[1].

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[1]Primary comparison for evaluating carcinogenicity is with the concurrent controls, performing laboratory controls are secondary; whereas, comparison with literature controls provides only tertiary information.

D. 2. Mouse Oncogenicity Study - Gulf South

Reference: NCI "Bioassay of Tetrachlorovinphos for Possible Carcinogenicity" at Gulf South Research Institute, Study # NCI-CG-TR-33, 1978.

Gardona was administered in the diet to groups of 50 male and 50 female B6C3F1 mice at 0, 8000 or 16,000 ppm for 80 weeks (with an additional observation period of 12 weeks). Matched controls (0 ppm) consisted of 10 untreated mice/sex; pooled controls were from bioassays of 4 other test chemicals (40 males and 40 females).

In male mice there was a statistically significant increase in hepatocellular carcinoma at both dose levels, with a statistically significant trend. There was also a liver lesion designated as "neoplastic nodule"[1] which was statistically significant in males, at the low dose only, and in females at both doses with a statistically significant trend. Tumor incidences are given in Table 4.

MTD: Previous discussion is relevant here too; that is, the MTD was apparently achieved or slightly exceeded at 8000 ppm.

Historical Controls
In treated male mice at both doses, the incidence of hepatocellular carcinoma was well above that reported in the literature for B6C3F1 male, however, the more pertinent historical control data from the performing laboratory were not available for making the secondary comparison (see footnote to page 4).

Deficiencies in study design were discussed; however, the results were considered to be supportive of the Hazleton mouse study.

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[1] This designation is puzzling; the term, neoplastic nodule (used by NCI/NTP until 1985) was recommended in 1975 to describe a spectrum of non-malignant proliferative lesions in the rat liver.

TABLE 4

Gulf South Mouse Study

Mouse Liver Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver	Males			Females		
	Pooled Control	Low Dose	High Dose	Pooled Control	Low Dose	High Dose
Carcinoma	5/49 (10)**	36/50 (72)**	40/50 (80)**	2/48 (4)	5/49 (10)	2/47 (4)
Neoplastic nodules	3/49 (6)	11/50 (22)**	2/50 (4)	1/48 (2)*	14/49 (29)**	9/47 (19)**

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose. Numbers in parentheses denote percent.

*p <0.05 **p <0.01

†Number of tumor bearing animals/number of animals at risk.

D. 3. Rat Oncogenicity Study - Gulf South Research Inst.

Reference: NCI "Bioassay of Tetrachlorovinphos for Possible Carcinogenicity" at Gulf South Research Institute, Study # NCI-CG-TR-33, 1978.

Gardona was administered in the diet to groups of 50 male and 50 female Osborne-Mendel rats at 0, 4250 or 8500 ppm for 80 weeks (with an additional observation period of 31 weeks). Matched controls (0 ppm) consisted of 10 untreated rats/sex; pooled controls were from bioassays of 4 other test chemicals (45 males and 45 females).

In female rats there was a statistically significant increase in thyroid C-cell adenoma at 8500 ppm (HDT), with a statistically significant trend. There was also a statistically significant increase in adrenal cortical adenoma at 8500 ppm, with a statistically significant trend. Tumor incidences are given in Table 5.

Male rats did not show treatment related increases in either of these tumors.

Historical Controls
The incidences of thyroid and adrenal adenomas in treated rats of both sexes were well within the literature reported incidences for Osborne-Mendel rats; the more pertinent control incidences from the testing laboratory were not available for comparison (see footnote to page 4).

Although there was an oncogenic response in this study, the Committee expressed concerns regarding the study design (the use of pooled controls).

TABLE 5

Gulf South Rat Study

Thyroid and Adrenal Tumor Rates† (%).
Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dietary Concentrations (ppm)			Historical Controls	
	0[a]	4250	8500	Mean	Range
Thyroid Tumors					
Males					
C-cell adenoma	2/46 (4)	2/45 (4)	3/45 (7)	(8.2)	(0-16)
Females					
C-cell adenoma	1/46 (2)*	2/50 (4)	7/46 (15)*	(20.4)	(2-31)
Adrenal Tumors					
Males					
Cortical adenoma	2/52 (4)	3/48 (6)	1/45 (2)	(14)	(2-22)
Females					
Cortical adenoma	0/50 (0)*	2/49 (4)	5/50 (10)*	(24)	(13-27)

Note: Significance of trend denoted at Control. Significance of pairwise comparisons with control denoted at Dose level. Numbers in parentheses denote percent.

[a]Pooled controls

*p <0.05.

4. Rat Chronic Toxicity Study - Tunstall Labs.

Reference: Shell Chemical Company "The Oral Toxicity of the Halophenyl Vinyl Phosphate Insecticide Gardona (SD 8447)" at Tunstall Laboratories, Study # T 507521/10, Nov. 1967.

Gardona was administered in the diet to groups of male and female Porton strain rats at 0, 5, 25, 125, or 2000 ppm for 2 years. A total of 60 rats/sex were used for the control group (0 ppm); 20 rats/sex for the 2000 ppm group; 40 rats/sex for all others.

There were no compound-related lesions reported.

Clinical chemistry measurements revealed the following significant findings, but only in the group dosed at 2000 ppm (HDT):

> Final body weight and food consumption were significantly lower than controls in both sexes; plasma and RBC cholinesterase activity (males and females, respectively) were significantly decreased; total serum protein and serum urea were also decreased in female rats.

Kidney weights of male rats at 2000 ppm were significantly lower than controls; liver weights of females at 2000 ppm were significantly higher than controls. There was no treatment-related effect on survival. The NOEL was established at 125 ppm for both sexes.

Although it was adequate as a chronic study (for which it was designed) the Committee agreed this study was deficient in design for an oncogenicity study (small number of animals).

E. Additional Toxicology Data on Gardona:

1. Metabolism

Limited data indicate that in rats and dogs Gardona is readily absorbed from the GI tract and distributed to different tissues. Gardona appears to be rapidly and (apparently) completely metabolized and excreted in the urine (mainly) and feces.

2. Mutagenicity

There were three unacceptable Gardona studies submitted by the Registrant.

In the dominant lethal test, although there were a couple of statistically significant decreases in the number of implants, these differnces were very small. The Committee felt this marginal response was suggestive, but not conclusive evidence and that the study should be repeated at higher doses. Further protocol and reporting deficiencies led to an unacceptable classification.

The Registrant has been asked to repeat all of the mutagenicity studies.

3. Developmental or Reproductive Effects

Gardona was not teratogenic to rabbits (but was fetotoxic) and did not demonstrate any adverse reproductive effects in a three-generation study in the rat.

4. Subchronic and Chronic Toxicity

In studies in the rat and dog, Gardona inhibited plasma, RBC, and brain cholinesterase activity; body weight gains were depressed; hemoglobin was reduced and absolute and/or relative liver weights were significantly increased.

E. 5. Structure-Activity Correlations

Gardona is structurally related to numerous organophosphate pesticides; Chlorfenvinphos, Crotoxyphos were both found to be mutagenic, but there is no information on carcinogenicity for either one. Dichlorvos (DDVP) was found to be both mutagenic and carcinogenic, inducing forestomach squamous cell tumors in both sexes of B6C3F1 mice; and leukemia in males and pancreatic adenomas in both sexes of Fischer 344 rats (these are different tumor sites than is the case with Gardona). DDVP was classified by the Peer Review Committee as a Group B2 carcinogen [Meeting, July 1, 1987, reconfirmed at meeting of Sept. 29[1].].

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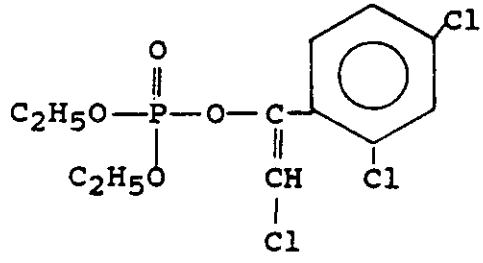
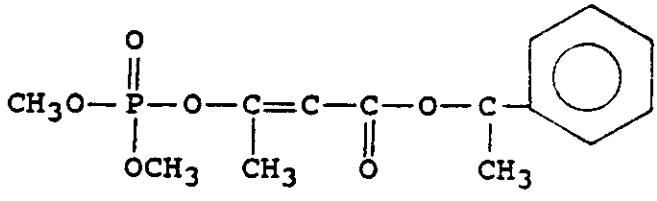
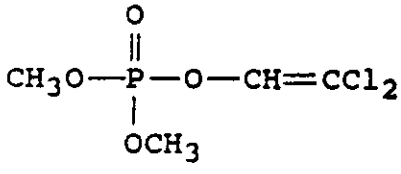
[1]SAP evaluated DDVP as a Group C carcinogen [Meeting, Sept. 23, 1987].



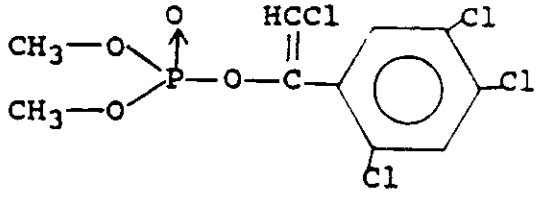
Chlorfenvinphos



Crotoxyphos



Dichlorvos



Gardona

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Gardona to be of importance in a weight-of-the-evidence determination of oncogenic potential.

In 2 separate studies in the B6C3F1 mouse, dietary administration of Gardona resulted in hepatocellular adenoma/carcinoma.

Study 1 (Hazleton):

In female mice, there was a statistically significant increased incidence of hepatocellular carcinoma at 8000 and 16,000 ppm and in combined adenoma/carcinoma at 1600, 8000 and 16,000 ppm; adenomas were statistically increased at 16,000 ppm only; there was a statistically significant trend for adenomas, carcinomas and for combined adenoma/carcinoma. In male mice there was a statistically significant increase in hepatocellular combined adenoma/carcinoma at 16,000 ppm, with a statistically significant trend.

In male mice, there was also a statistically significant increase in the incidence of kidney adenoma, carcinoma and of combined adenoma/carcinoma at 16,000 ppm, with a statistically significant trend.

Study 2 (Gulf South):

There was a statistically significant increase in hepatocellular carcinoma, with a statistically significant trend at 8000 and 16,000 ppm in male mice. There was also a statistically significant increase in "neoplastic nodules" at 8000 ppm in males, and at 8000 and 16,000 ppm in females (in females there was also a statistically significant trend). Deficiencies in the study were noted; however, the data were considered supportive of the Hazleton study results.

There was an MTD issue, in terms of the liver tumors: the Committee determined that at 8000 ppm (in the Hazleton study), the MTD was achieved or slightly exceeded; however, at 16,000 ppm it was exceeded, based on severe liver necrosis (even though this apparently was not life-threatening, as survival was actually increased at this dose!). The sponsor maintains that the MTD was exceeded at 8000 ppm, and therefore effects above 1600 are not "experimentally valid"; nevertheless there was a statistically significant increase in the incidence of hepatocellular combined adenoma/carcinoma in female mice, even at 1600 ppm.

F. Weight of Evidence (continued)

As for the kidney tumors, it was noted that even if the MTD was exceeded at 16,000 ppm, the kidney was not a target organ for toxicity; however, it was also noted that saturation of metabolic processes in the liver could still have been a significant factor with regard to the kidney tumors.

In the Gulf South Study in the Osborne-Mendel rat, dietary administration of Gardona was associated with a statistically significant increase in thyroid C-cell adenoma and adrenal cortical adenoma, with a statistically significant trend, in female rats at 8500 ppm, (HDT). Deficiencies in study design were noted and the evidence was judged to be equivocal (suggestive at best).

Another chronic rat study was performed in the Porton strain rat, and it was negative for oncogenicity; however, the HDT (2000 ppm) was not considered to be high enough, and only a small number of animals (20) were tested. The Committee concluded that it was acceptable as a chronic study, but not as an oncogenicity study.

Mutagenicity studies on Gardona were unacceptable due to study deficiencies; however, positive activity in a dominant lethal assay (<u>also unacceptable</u>) was noted.

Gardona is structurally related to numerous organophosphate pesticides. Dichlorvos (DDVP) was mutagenic in several assays and oncogenic in both sexes of mice and rats. Chlorfenvinphos and Crotoxyphos are both mutagenic, but their carcinogenic potential has not been determined.

There were no reproductive effects in the rat, but there was developmental toxicity (fetotoxicity) in the rabbit.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee discussed the evidence summarized in Section F with regard to both a B2 and a C classification.

A B2 was considered, but rejected because it was concluded that good evidence in 2 species was lacking, in that the rat (2nd species) study was equivocal, although suggestive evidence. Ancillary data: a suggestion of mutagenicity (from an unacceptable study) and SAR, based on DDVP (although there may be important differences in metabolism) could not provide sufficient support to warrant a B2 classification.

The mouse liver response in the Hazleton study was considered convincing, in itself. The NCI (Gulf South) mouse study also provides supporting evidence. The kidney response in male mice (Hazleton study) could not be completely ruled in or out.

The Committee concluded that the weight-of-the-evidence for Gardona was not strong enough to support a B2 classification, and agreed that Gardona should be classified as a C (possible human carcinogen, based on limited evidence in animals); it was recommended that a quantitative assessment of human risk be performed, based on the total mouse liver tumors.

The Peer Review Committee also concluded that the Registrant must submit another oncogenicity study in the rat.

Gardona ADDENDUM

Note to pages 3a, 3b, 5a and 6a:

Tables 1 and 3 and accompanying statistical analyses were provided by Ms. B. Fisher in the Qualitatitive Risk Assessment (QRA) Memo, 11/13/87.

Table 2 and accompanying statistical analyses were provided by Ms. Fisher, 8/27/87 (appended) from summary data provided by Dr. M. Ioannou.

Tables 4 and 5 and accompanying statistical analyses were provided by Dr. Ioannou from the NCI study "Bioassay of Tetrachlorvinphos For Possible Carcinogenicity" NCI-CG-TR-33.
These data were not analyzed by the Tox Branch Statistics Team, and thus were not included in the QRA.

Note to page 4:

In the Hazleton Mouse Study, "...female mice showed a significant decrease in mortality with increasing doses of Gardona" and "male mice exhibited a decrease in mortality with increasing dose of Gardona" [QRA, 11/13/87].

13a

8/27/87

Gardona - Mouse Study - Male Liver Tumor (Carcinoma +/or Adenoma) Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Liver	0	17.5	64	320	1600	8000	16000
Carcinoma	26/113 (25)	17/58 (29)	16/58 (28)	10/51 (20)	14/55 (25)	13/60 (22)	22/59 (37)
Adenoma	2/80 (2)	1/37 (3)	0/42 (0)	0/35 (0)	1/39 (3)	5/47 (11)	3/47 (6)
Carcinoma and/or Adenoma	~~28/112~~ 113 (25)*	18/58 (31)	16/58 (28)	10/51 (20)	15/55 (27)	18/60 (30)	25/59 (42)*

first-Ad - wk 99 in control

first-Ca - in wk 66 in 16,000 ppm

13544

R197101

Chemical Name: Tetrachlorvinphos

PC Code: 083701
HED File Code: 21200 Peer Review
Memo Date: 4/14/1988
File ID: TX0055263
Accession #: 000-00-0137

HED Records Reference Center
2/9/2012